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Report No. 3

"MICROSTIMULATION OF THE LUMBOSACRAL SPINAL CORD"

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This QPR is being sent to you before it has been reviewed by the staff of the Neural Prosthesis Program.

Abstract

In this quarter, we conducted one experiment aimed at examinating the effects of chronic electrical stimulation on spinal cord tissue. Acute pulsing of the electrodes 21 days after implantation produced significant, but incomplete, voiding. The electrodes were pulsed chronically after 55 days of implantation. The results were similar to previous experiments and indicated that pulsing of the electrodes with continuous stimulation (20 Hz, 80 μA, 400 μsec/phase, 32 nC/phase, 3,200 μC/cm^2) applied in two 12-hour periods caused minimal depression of neuronal excitability.

Histologic analysis, while not complete, indicated that chronic pulsing of electrodes implanted in the sacral spinal cord induced only slight changes in the neural tissue compared to electrodes implanted for similar periods but not pulsed. The results support our previous observations that electrical stimulation may attract inflammatory cells.

We also report the results of two acute experiments during which complete bladder/urethral pressure profiles were obtained during passive (no stimulation) and active (spinal cord stimulation) trials. In some instances, stimulation induced a negative pressure at the level of the bulbo- and ishiocavernosus muscles. This may be the source of the stimulation-induced urethral pressure decrease noted in earlier experiments. Results also demonstrated that the urethral pressure profile is influenced significantly by the bladder background pressure.

Introduction

The overall goals of this contract are to develop a method of chronic microstimulation of the sacral cord of the cat to effect micturition, and to evaluate the effects of the electrical stimulation on neural and surrounding tissues. In this report we discuss the results from one chronic experiment in which microelectrodes implanted in the spinal cord for 55 days were stimulated continuously for 12 hrs on each of 2 successive days. Electrode impedance measurements remained stable throughout this period. Physiologic results demonstrated that this prolonged stimulation only slightly depressed neuronal excitability. This animal demonstrated good micturition in response to stimulation of the spinal cord 21 days after electrode implantation. However, repeated tests of the residual volume demonstrated that the stimulation-induced micturition amounted to 32-52% of the initial volume. Preliminary histologic results are reported here and final results will be reported in the next QPR.

Two acute experiments were performed in which a constant velocity motor was used to slowly withdraw a solid-state pressure sensor from inside the bladder along the complete length of the urethra. Preliminary findings indicate that this technique provides repeatable bladder/urethral pressure profiles in both passive (non-stimulation) and active (stimulation of the spinal cord) trials. These efforts have been hampered by the malfunction of our data recorder which forced us to return to our previous strip chart recording methodology and subsequent slow data processing.

Methods

Chronic Experiment. An adult male cat (SP-65) was anesthetized with 50% nitrous oxide and 1-2% Halothane and the spinal cord exposed as described previously. The S_2 region of the spinal cord was localized by stimulation of the dermatome it serves while recording the dorsal cord potential supradurally, as described previously. Four activated iridium microelectrodes (50 μm dia., 2.8 mm long, 2000 μm^2 exposed stimulating surface) were implanted manually at approximately the dorsal midline of the spinal cord and angled laterally at about 10 degrees. No matrix was used to support the electrodes. The electrodes were pulsed individually and the bladder lumen pressure and intraurethral tone were monitored. The electrodes were advanced into the cord until good elevation of bladder pressure was produced by the stimulation. The dura was then closed over the electrodes and the effect of the stimulation again measured. A silastic

pad (to which the stimulating electrode leads had been glued) was sutured to the dura to reduce traction on the electrodes. The ground (indifferent) electrode was sutured in place over the microelectrodes. A small hole was made in the dura and a recording electrode inserted so as to lie approximately 3 cm caudal to the stimulating electrodes. A suture was used to secure the recording electrode to the dura. A reference electrode was sutured to the muscle 2 cm above the dura. The wound was flushed with antibacterial solution and the muscle and skin were closed in layers. Subsequent recordings were made with the animal anesthetized with Pentothal (i.v., as needed) or Nembutal (i.v., as needed). A sterile catheter and sterile saline were used during recording of the bladder lumenal pressure.

After functional electrodes had been implanted for 55 days, the cat was anesthetized with Propofol (0.5-2.0 ml/hr) and chronic stimulation was initiated. This protocol called for continuous stimulation for 12 hours/day for two consecutive days. The animal's core temperature was monitored and maintained at approximately 38 C using a heating pad and radiant heat lamp. Normal saline or lactated ringers solution was administered (iv, as needed) to maintain adequate hydration.

Acute Experiments. Two adult male cats were anesthetized with 50% nitrous oxide and 1-2% Halothane. The spinal cord was exposed using a standard dorsal laminectomy. In all cases, the S_2 region was localized in the manner described above. The spinal cord was covered with light mineral oil to prevent drying. The stimulating electrodes were inserted into the cord using a standard stereotaxic apparatus.

A small (transducer ~1.3 mm dia, catheter ~0.8 mm dia) pressure transducer (Millar Instruments) was passed along the length of the urethra and then slowly withdrawn at a constant rate using an electric motor. The frequency response of this pressure transducer is flat to 10 KHz. A potentiometer coupled to the shaft of the motor allowed measurement of the distance the catheter was withdrawn as a function of time. Both the pressure and position signals were digitally stored on tape (18.5 KHz sampling rate per channel). These signals were then digitized off-line at 10 Hz per channel for further analysis. The experimental setup is shown in Figure 1.

Histology. Within 20 minutes of the end of an experiment the animal was anesthetized with Nembutal and perfused through the aorta with saline followed by 2 L of 1/2 strength Karnovsky's fixative (2% paraformaldehyde and 2.5% glutaraldehyde) in 0.1 M sodium

cacodylate buffer, pH 7.4. With the electrodes *in situ*, the complete cord and spinal roots were dissected out to precisely localize the microelectrodes. Two-mm-thick transverse sections containing the electrode tracks were dissected, processed and embedded in epoxy resin. One-μm thick sections were cut serially through the blocks and examined using light microscopy.

Results

The results from the chronic experiment (SP-65) were quite good. Of the four electrodes implanted, three demonstrated good evoked potentials and all showed consistent impedance over the course of the 55 day implantation period (Figure 2). This implant array was able to evoke significant voiding using a pulse amplitude of 80 μA and pulse width of 400 μsec/phase delivered at 20 Hz with a duty cycle of 1 s of stimulation and 2 s of no stimulation. No voiding occurred prior to initiating the stimulation and when it was terminated voiding often continued for 2-3 s before stopping. Voiding could typically be started and stopped several times before the volume decreased to a level at which the stimulation was no longer effective.

In four successive trials, the bladder was initially drained and then filled with 25 ml of saline. This volume resulted in a moderately full bladder as judged by manual palpation through the abdominal wall. Stimulation as described was used to initiate voiding and then residual volume was measured. The results are shown in Figure 3. The voided saline comprised 36%, 32%, 40%, and 52% of the initial volume.

After the electrode array had been implanted for 55 days, electrodes 2 and 4 were stimulated continuously at 20 Hz, 80 μA, 400 μsec/phase, 32 nC/phase, 3,200 $\mu C/cm^2$ (cathodic first) for 12 hours on each of two successive days. The other electrodes served as unpulsed controls. Figure 4 shows the amplitude of the average evoked compound action potential (AECAP) as a function of the stimulus amplitude for electrode 2 and Figure 5 shows the results for electrode 4. The curves were generated before the start of the first 12 hour stimulation period and at the start and end of the second 12 hour period of stimulation. The results for electrode 2 did not indicate evidence of depressed neuronal excitability. In the case of electrode 4 there may have been a slight depression of neuronal excitability. Overall, neuronal excitability showed little difference before, during, or after the prolonged stimulation period.

Histologic results from SP-65 showed that the four electrodes were implanted within about 3 mm of each other in the S_2 segment. The electrodes were positioned in a rostral-caudal order: 3, 2, 1 and 4, with the caudal three electrodes close together. Electrode 3 (not pulsed) was implanted in the rostral part of the S_2 segment as identified by the shape of the lateral horn. The electrode passed medially, through the preganglionic parasympathetic nucleus and into the ventrolateral ventral horn. Tissue surrounding the tip of the electrode will be examined with electron microscopy. The epi-pial spherical bead of this electrode depressed the cord to a depth of 0.5 mm (Fig. 6).

Electrode 4 (pulsed) passed from medial to lateral and into the medial intermediate gray, with its tip in the ventral horn (Fig. 7). The dorsal surface of the cord was not depressed as in the case of electrode 3. By light microscopy, the tissue surrounding electrode 4 (pulsed) looked similar to that surrounding electrode 3 (unpulsed). There was cellular debris adjacent to the tracks, but there were intact neurons nearby. An aggregation of macrophages was identified ventral to the tip of electrode 4. Electron microscopy of the site of the tip of electrode 4 revealed many dark neutrophils, macrophages, small necrotic cells, and neurons with polymorphic nuclei (Figs. 8 and 9). Blood vessels contained, and were surrounded by, mononuclear cells and by polymorphonuclear leukocytes (Fig. 10). Necrotic areas were filled with astroglial filaments (Fig. 11).

The remaining electrode tracks have yet to be sectioned.

In an acute experiment (SP-64), a small pressure transducer was inserted into the bladder and withdrawn slowly (1.54 cm/s) using a constant velocity motor. The computer recorded the pressure and the voltage across a potentiometer linked to the shaft of the motor. Figure 12 is an example of a single trial demonstrating the form in which the raw data is collected. Figure 13 displays six successive passive (no stimulation) bladder/urethral pressure profiles as a function of distance from the tip of the penis. Figure 14 shows five successive active (stimulation of the spinal cord) bladder/urethral pressure profiles. Unfortunately, these two sets of data were recorded without control of the background bladder pressure, making exact comparison difficult. The most notable feature in Figure 14 is the negative pressure approximately 3 cm from the tip of the penis. This corresponds to the position of the bulbocavernosus and ishiocavernosus muscles.

Two other acute experiments were conducted to examine this response further. However, the data from SP-66 were lost due to an intermittent malfunction of the data recorder. This also affected SP-67 to the extent that only data recorded on a strip chart recorder were obtained. Results are shown in Figure 15 in which the background bladder pressure was low (C), medium (B), and high (A) and the catheter was withdrawn as indicated (D). In each case, a passive trial is shown in the left trace and an active trial is shown in the right trace. In all active trials the stimulation was 80 μA, 400 μsec/phase delivered at a continuous rate of 12 Hz. The onset of the stimulation is indicated by the arrows.

No voiding occurred during any of the passive trials regardless of the background bladder pressure, even when the catheter had been fully removed from the urethra. In the active trials, stimulation that was initiated when the bladder background pressure was high (A) produced a small amount of voiding around the catheter as it was being withdrawn and then a larger amount when the catheter had completely exited the urethra. During trials in which the bladder background pressure was low or medium, the stimulation did not produce voiding.

It is interesting that the urethral pressure profile changed significantly with bladder background pressure. The pressure presumably exerted by the external urethral sphincter was dramatically reduced at higher bladder pressure. This was true during both the passive and active trials and is likely the result of reflex induced relaxation of the EUS.

Histologic results from SP-67 indicated that the acute electrode passed through the right dorsal columns and dorsal horn, through the right PPN in the intermediate gray and through the ventral horn into the ventral columns (Fig. 16). Several of the neurons appeared very dark and vesiculated cells and axons characteristic of acute damage were present (Fig. 17). Dissection of the sacral cord verified that this electrode was at the rostral end of the S_2 segment.

Discussion

The results from the chronic cat are encouraging. Good stimulation-induced voiding was seen three weeks after implantation. However, such voiding was incomplete, suggesting that bladder voiding evoked by spinal cord stimulation may rely on or be aided by reflexes that are most pronounced when the bladder is quite full. Several strategies could be employed to make voiding more complete including increasing the stimulation amplitude or frequency as the bladder

is emptied. After nearly two months of implantation the electrode array remained fully functional giving rise to large stimulation-induced bladder pressure increases. Good evoked potentials were obtained from three of the four electrodes. The cat showed no overt signs of behavioral or functional deficits throughout the implantation period. Despite two 12 periods of continuous stimulation at what we would consider functional levels, neuronal excitability was effected only slightly.

The data from the two electrodes, one pulsed and one not, although not yet complete, supports our previous observations that electrical stimulation within the spinal cord attracts inflammatory cells.

Comparing urethral pressure profiles during passive (no stimulation) and active (with spinal cord stimulation) trials demonstrated that in active trials, a significant negative pressure can occur at approximately the level of the bulbo- and ishiocavernosus muscles. This may be related to the decrease in urethral pressure noted in previous experiments which we attributed to relaxation of the EUS. However, this negative pressure was not seen in all animals. Urethral pressure profiles also demonstrated that significant differences could occur with changes in the bladder background pressure. Our results indicated that the response of the EUS is nearly eliminated when the bladder becomes quite full.

Publications

Woodford, B.J., Carter, R.R., McCreery, D.B., Bullara, L.A., Agnew, W.F. "Histopathologic and physiologic effects of chronic implantation of microelectrodes in sacral spinal cord of the cat." *Journal of Neuropathology and Experimental Neurology*, Vol. 55, No. 9, September 1996.

Future Work

In the next quarter we plan to continue acute experiments to further examine the effects of stimulation and pharmacological agents along the entire bladder/urethral length. We are presently working on a morphometric analysis system to determine if neurons are lost after chronic microelectrode implantation and whether this is influenced by chronic stimulation. We also plan to examine chronically stimulated tissue after 1-4 weeks of recovery.

EXPERIMENTAL SETUP

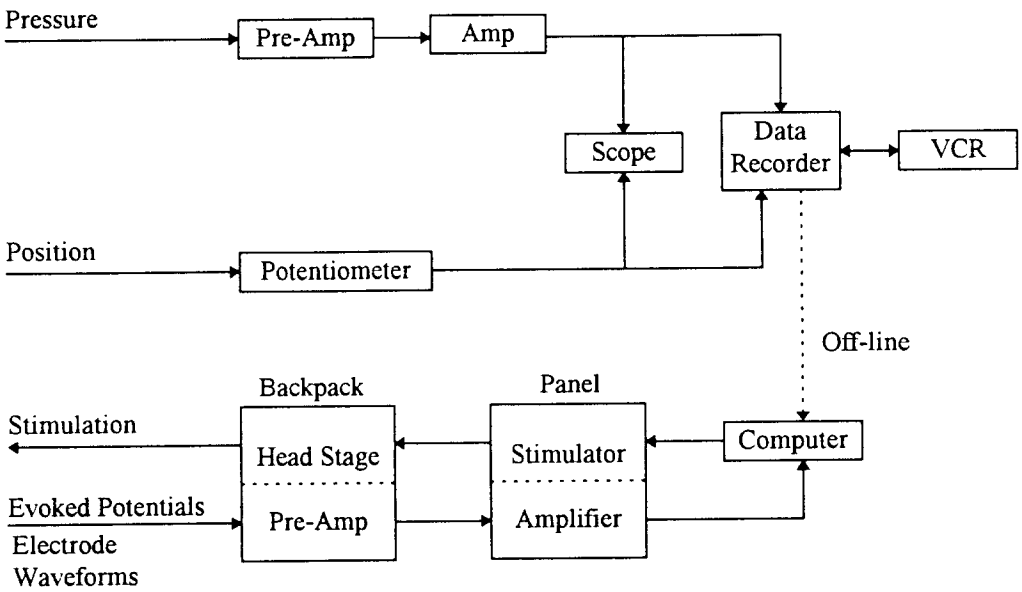


Fig. 1

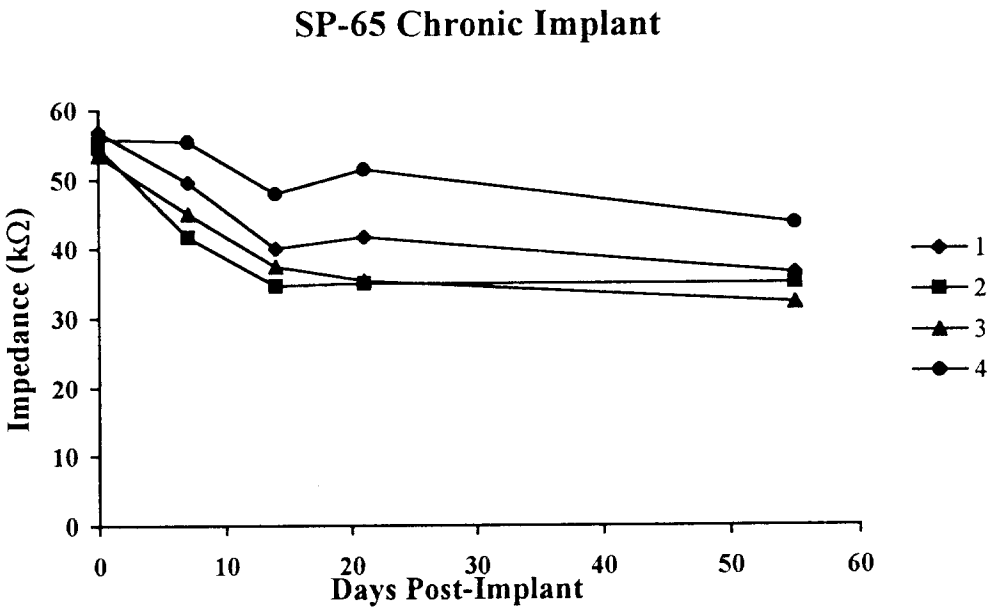

SP-65 Chronic Implant
Impedance (kΩ)
60
50
40
30
20
10
0
0
10
20
30
40
50
60
Days Post-Implant
1
2
3
4

Fig. 2

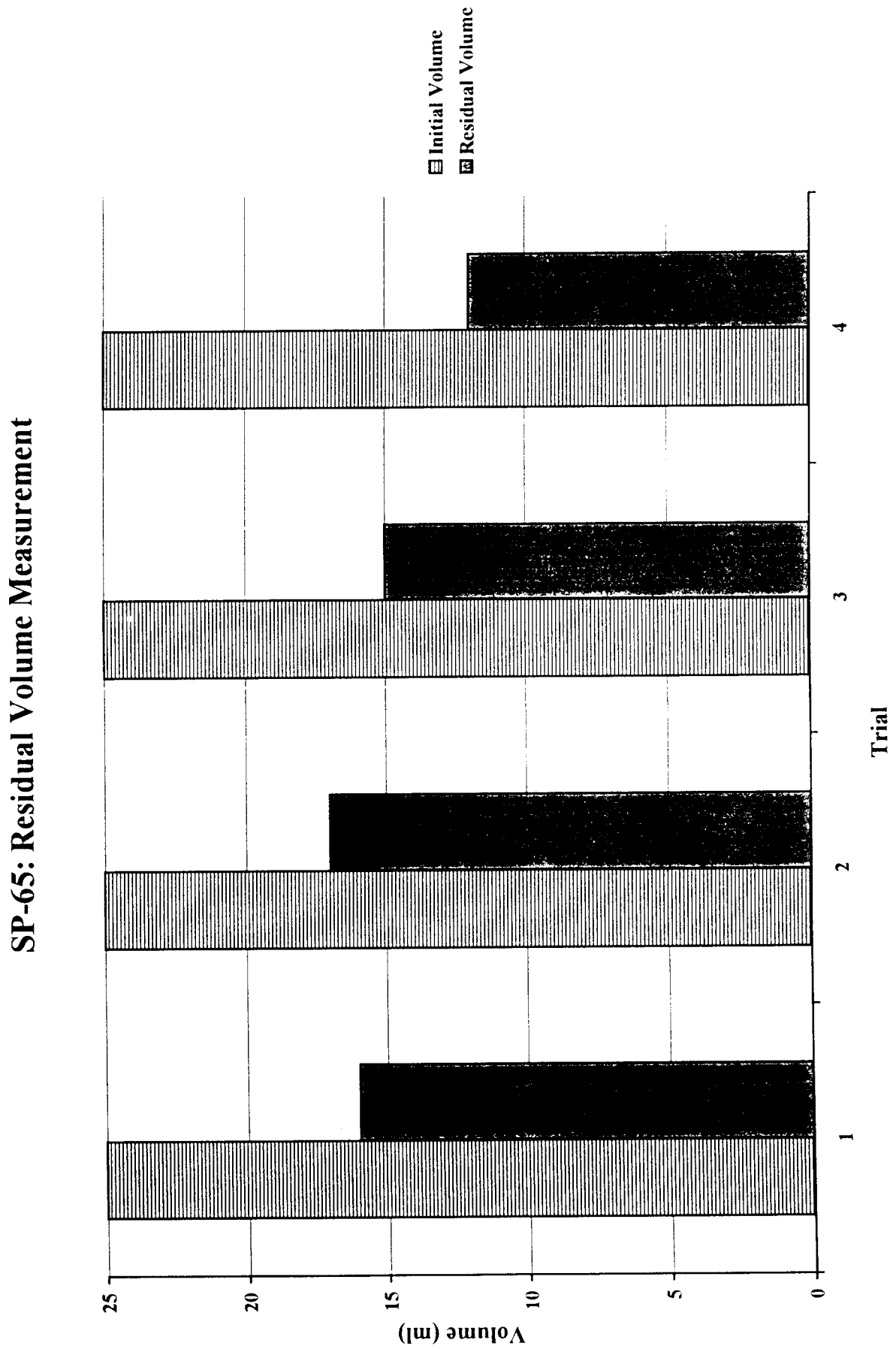

SP-65: Residual Volume Measurement
Initial Volume
Residual Volume
Volume (ml)
0
5
10
15
20
25
1
2
3
4
Trial

Fig. 3

SP-65

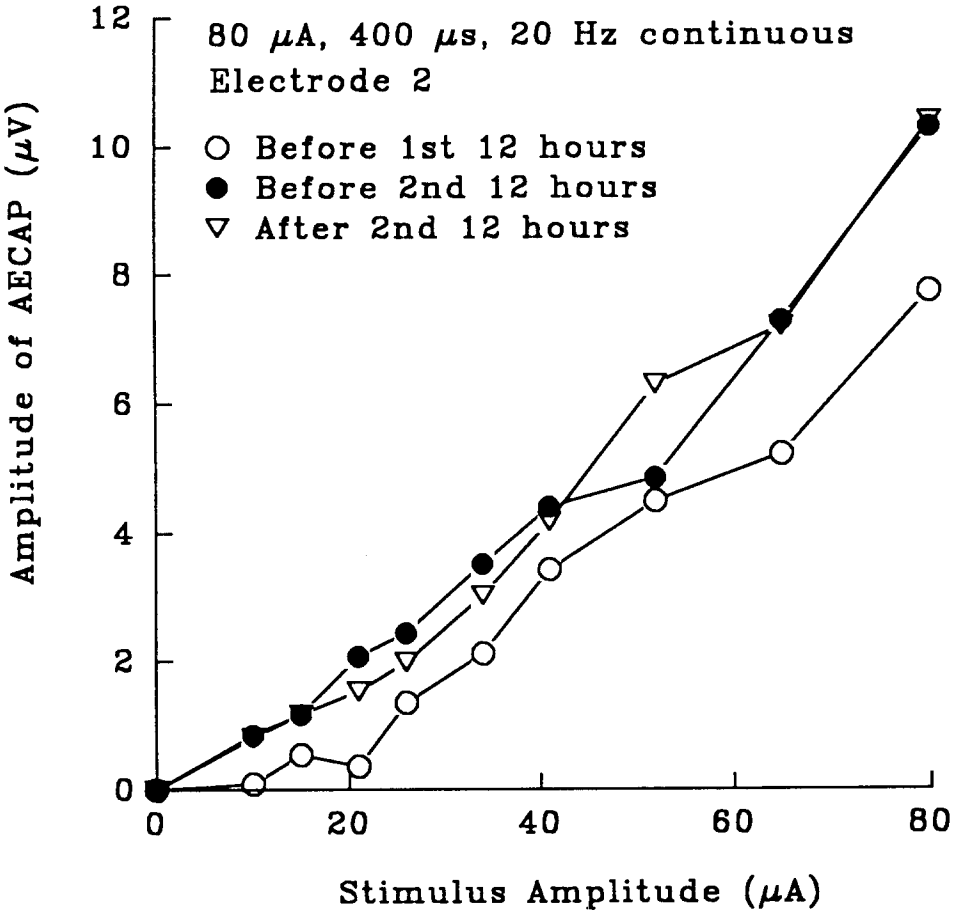

80 μA, 400 μs, 20 Hz continuous
Electrode 2
○ Before 1st 12 hours
● Before 2nd 12 hours
▽ After 2nd 12 hours
Amplitude of AECAP (μV)
12
10
8
6
4
2
0
0
20
40
60
80
Stimulus Amplitude (μA)

Fig. 4

SP−65

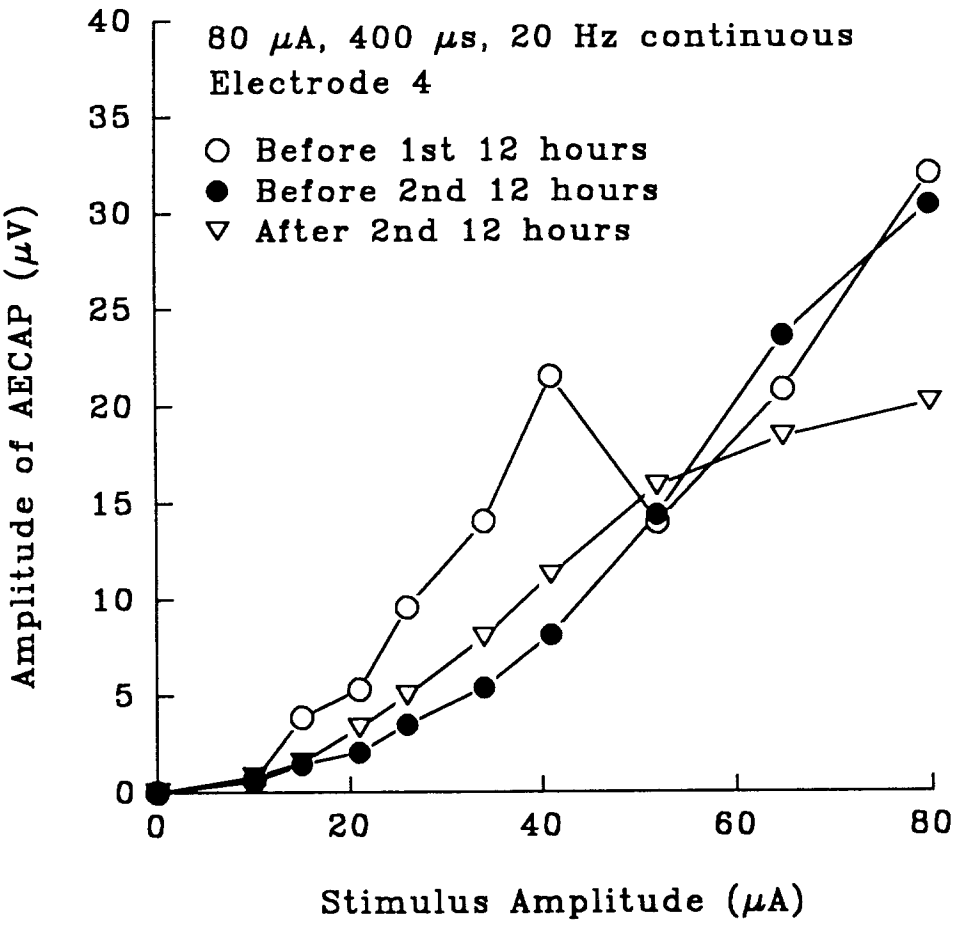

80 μA, 400 μs, 20 Hz continuous
Electrode 4
Before 1st 12 hours
Before 2nd 12 hours
After 2nd 12 hours
Amplitude of AECAP (μV)
Stimulus Amplitude (μA)
0
5
10
15
20
25
30
35
40
0
20
40
60
80

Fig. 5

Fig. 6. Animal SP-65. One μm thick plastic section through the S2 segment of the spinal cord. Note the 0.5 mm deep defect (asterisk) left by the spherical head of Electrode #3. An upper segment of the track (arrows) is seen passing through the dorsal horn. This and all succeeding micrographs were taken through the S2 segments of the spinal cord of animals SP-65 and SP-67. Bar = 250 μm.

Fig. 7. Animal SP-65. One μm thick plastic section through the track (T) of Electrode #4. The track passes through the right dorsal surface of the S2 segment of the spinal cord, through the left medial portion of the intermediate gray and terminates in the left ventral horn. Bar = 250 μm.

Fig. 8. Animal SP-65. Ultrathin section through the tip area of Electrode #4. The area consists of dark neutrophils (asterisk), macrophages (M) and small necrotic cells. Bar = 2 μm.

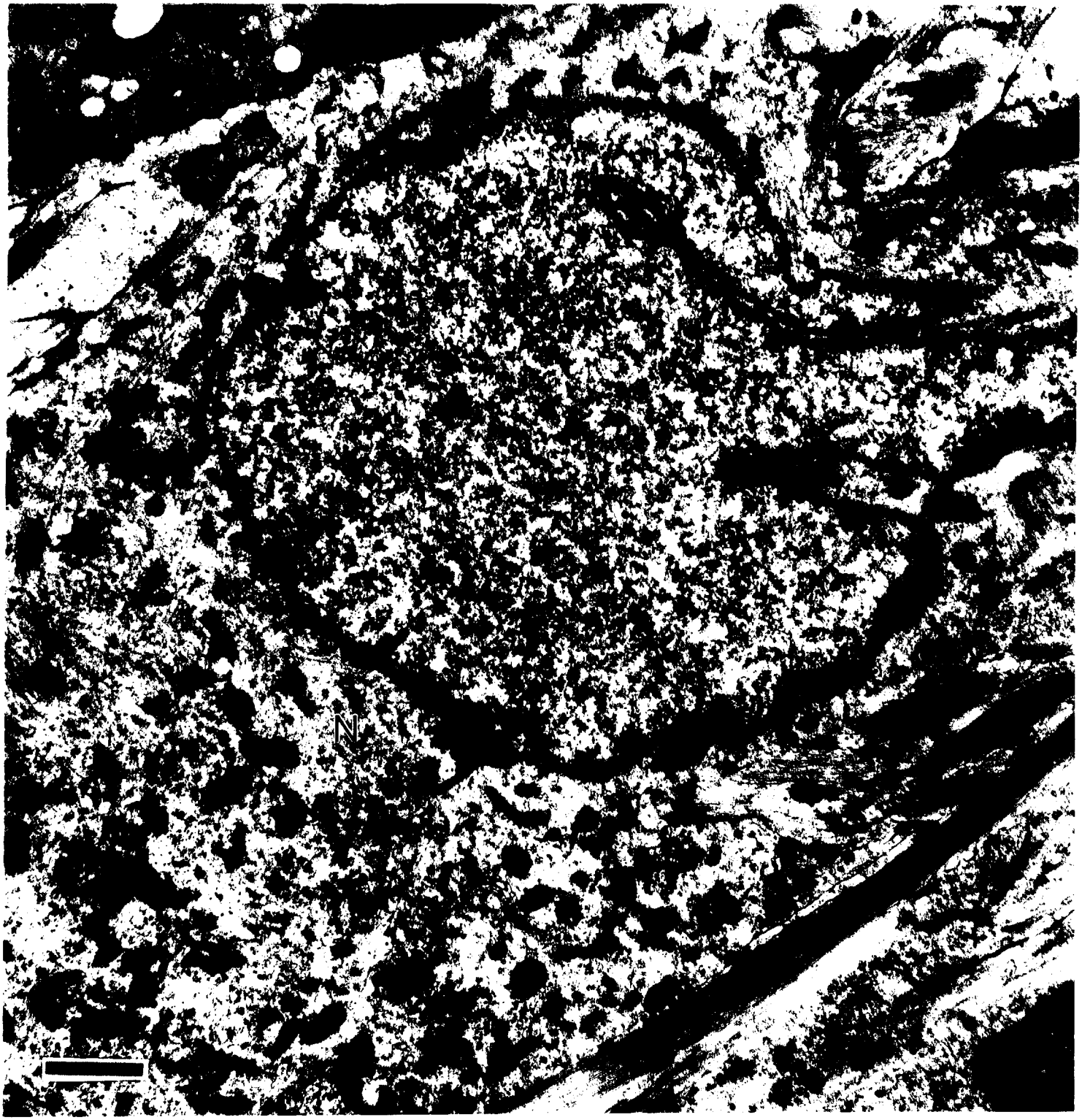


Fig. 9. Animal SP-65. Ultrathin section through same area as that shown in Fig. 8. Aside from the polymorphic nucleus, the neuron (N) appears normal. Bar = 1 μm.

Fig. 10. Animal SP-65. Ultrathin section showing a blood vessel near the tip of Electrode #4. An intraluminal neutrophil is present. Other neutrophils (asterisks) and mononuclear cells (M) lie close to the blood vessel. Some nearby axons (arrows) show deranged myelin. Bar = 1 μm.

Fig. 11. Animal SP-65. Ultrathin section showing an area near that shown in Fig. 10. This area is filled with necrotic debris admixed with astroglia and their abundant cytoplasmic fibrils (asterisks). Bar = 1 μm.

Fig. 12

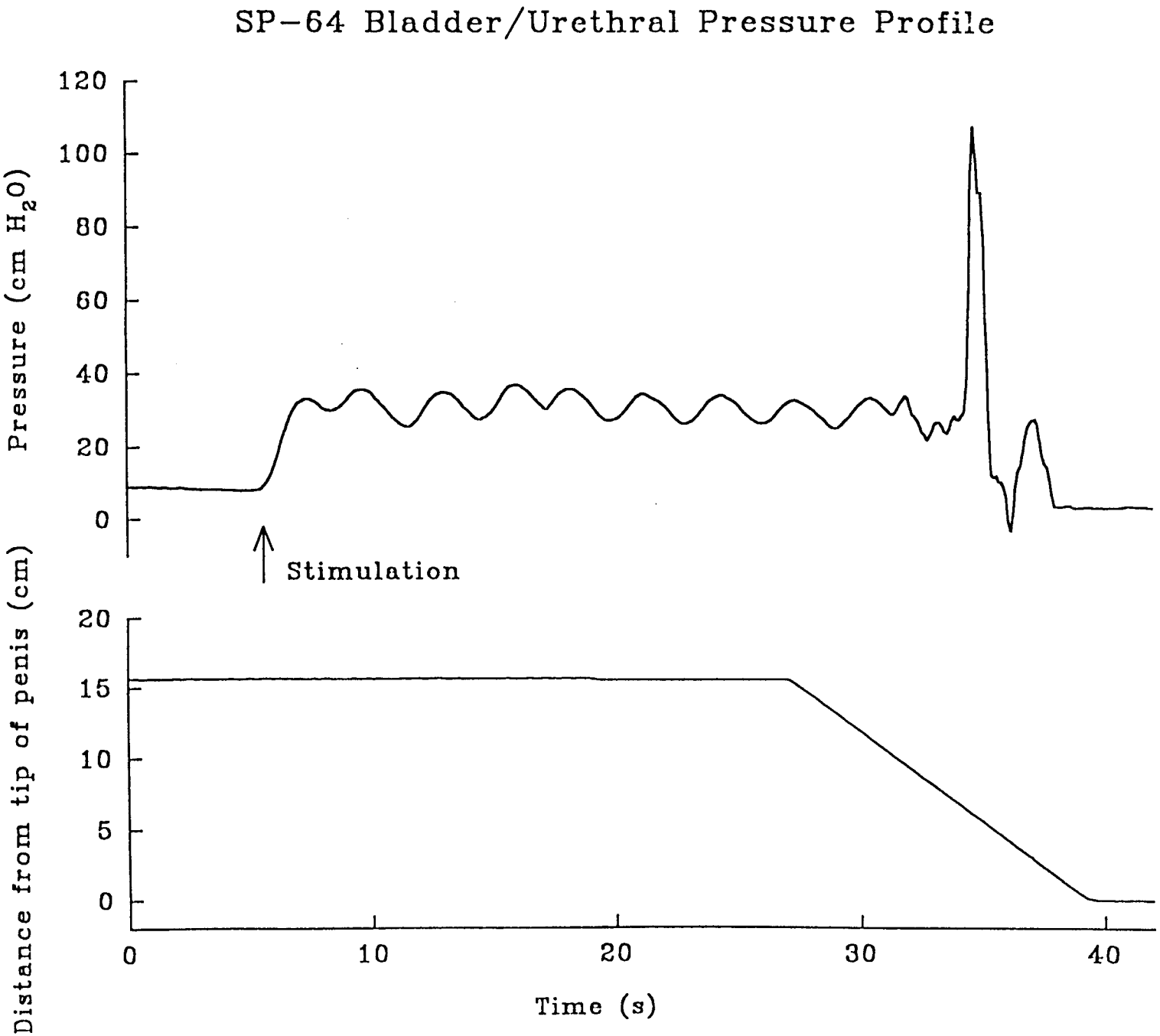

SP-64 Bladder/Urethral Pressure Profile
Pressure (cm H_2O)
120
100
80
60
40
20
0
Stimulation
Distance from tip of penis (cm)
20
15
10
5
0
0
10
20
30
40
Time (s)

Fig. 13

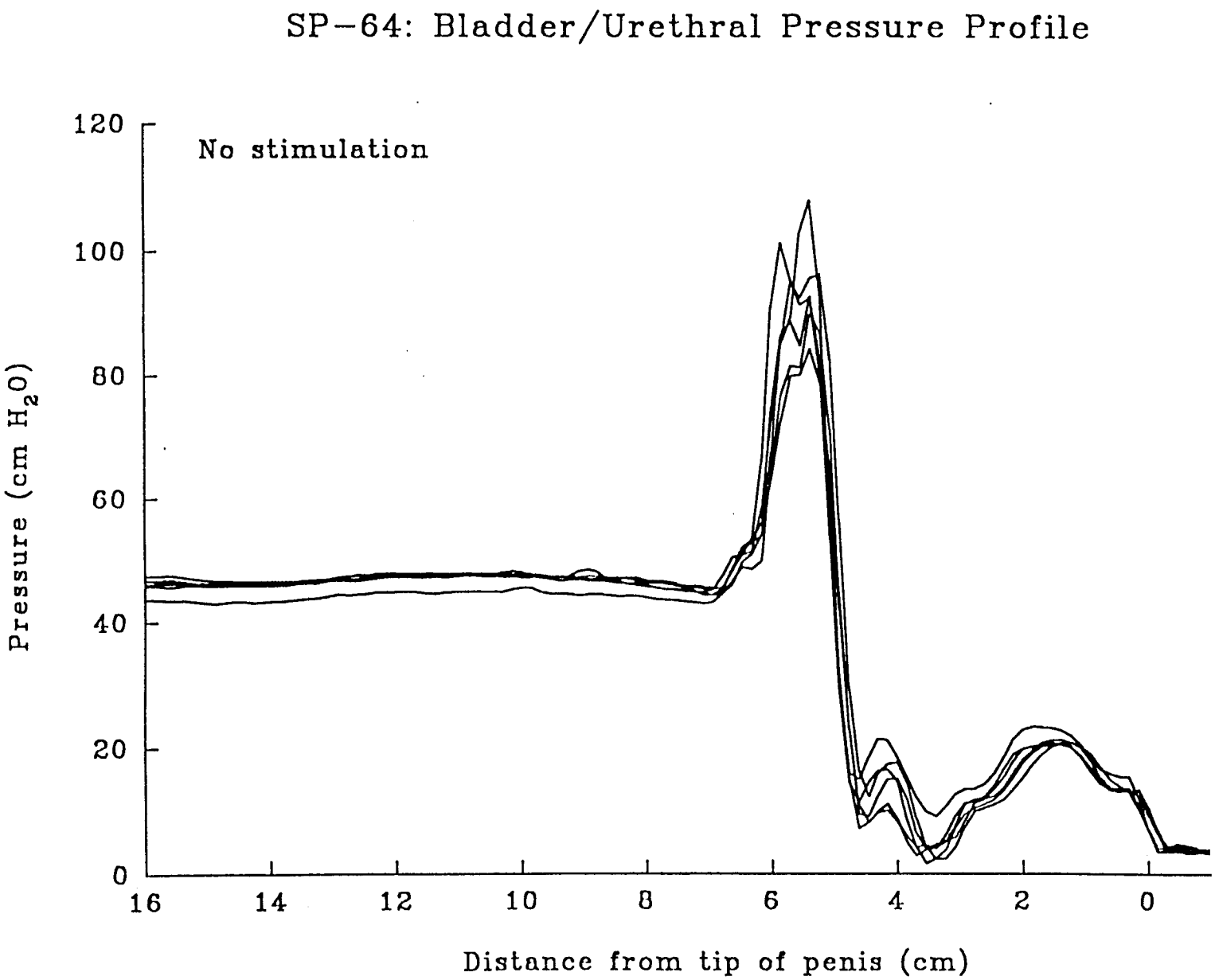
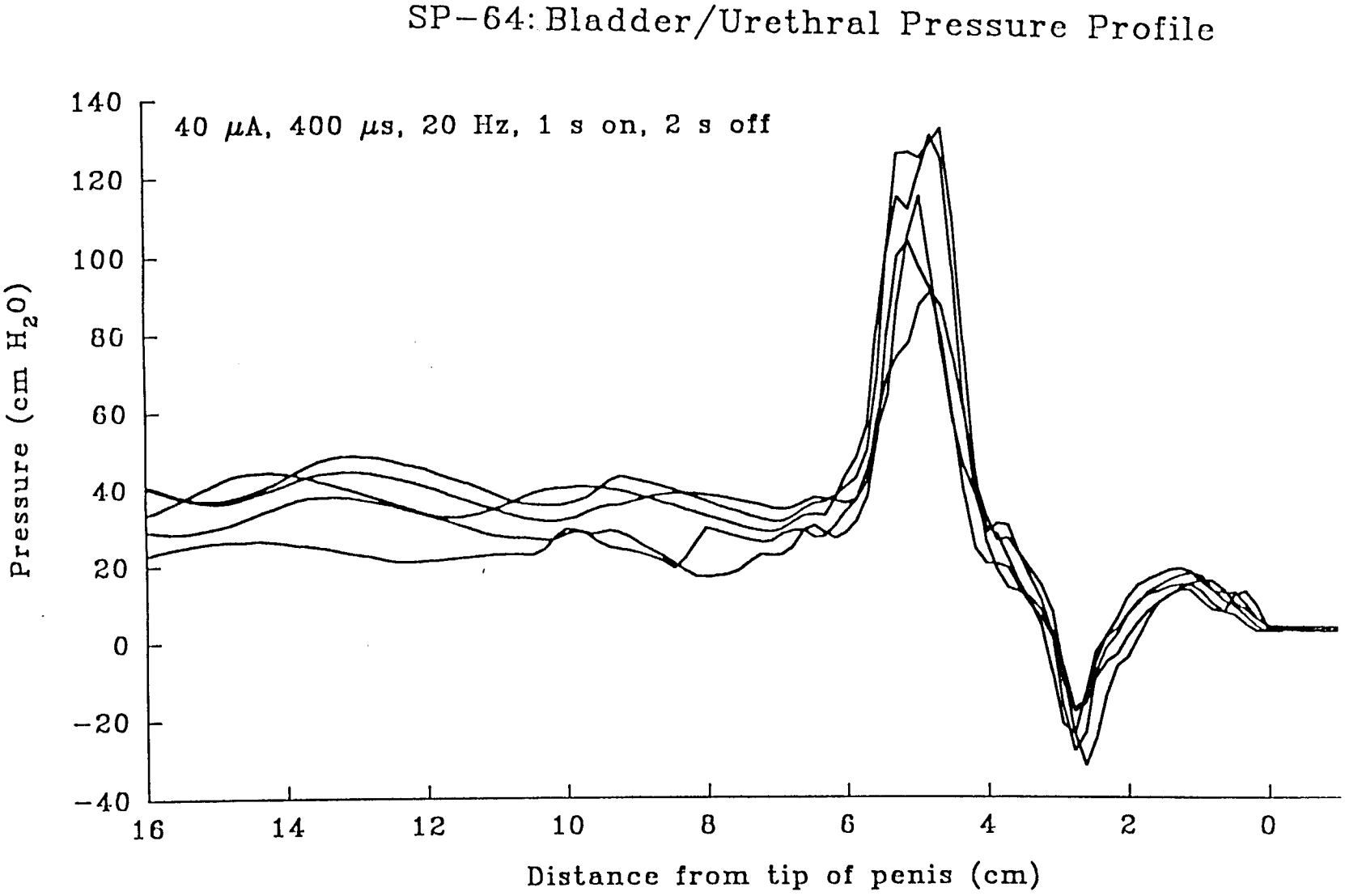
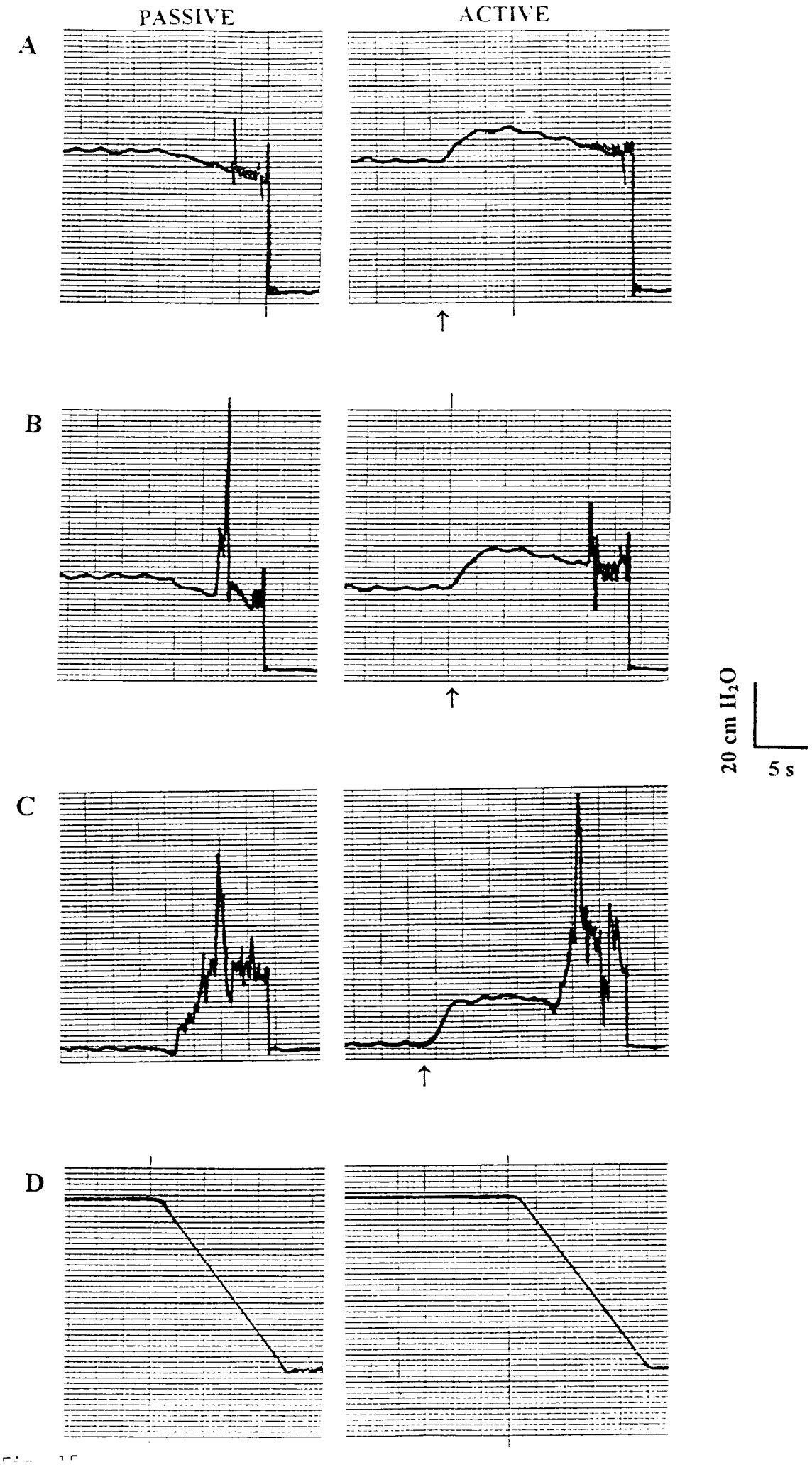

SP-64: Bladder/Urethral Pressure Profile
No stimulation
120
100
80
60
40
20
0
Pressure (cm H_2O)
16
14
12
10
8
6
4
2
0
Distance from tip of penis (cm)

Fig. 14


SP-64: Bladder/Urethral Pressure Profile
40 μA, 400 μs, 20 Hz, 1 s on, 2 s off
Pressure (cm H_2O)
140
120
100
80
60
40
20
0
-20
-40
16
14
12
10
8
6
4
2
0
Distance from tip of penis (cm)


PASSIVE
ACTIVE
A
B
C
D
20 cm H2O
5 s

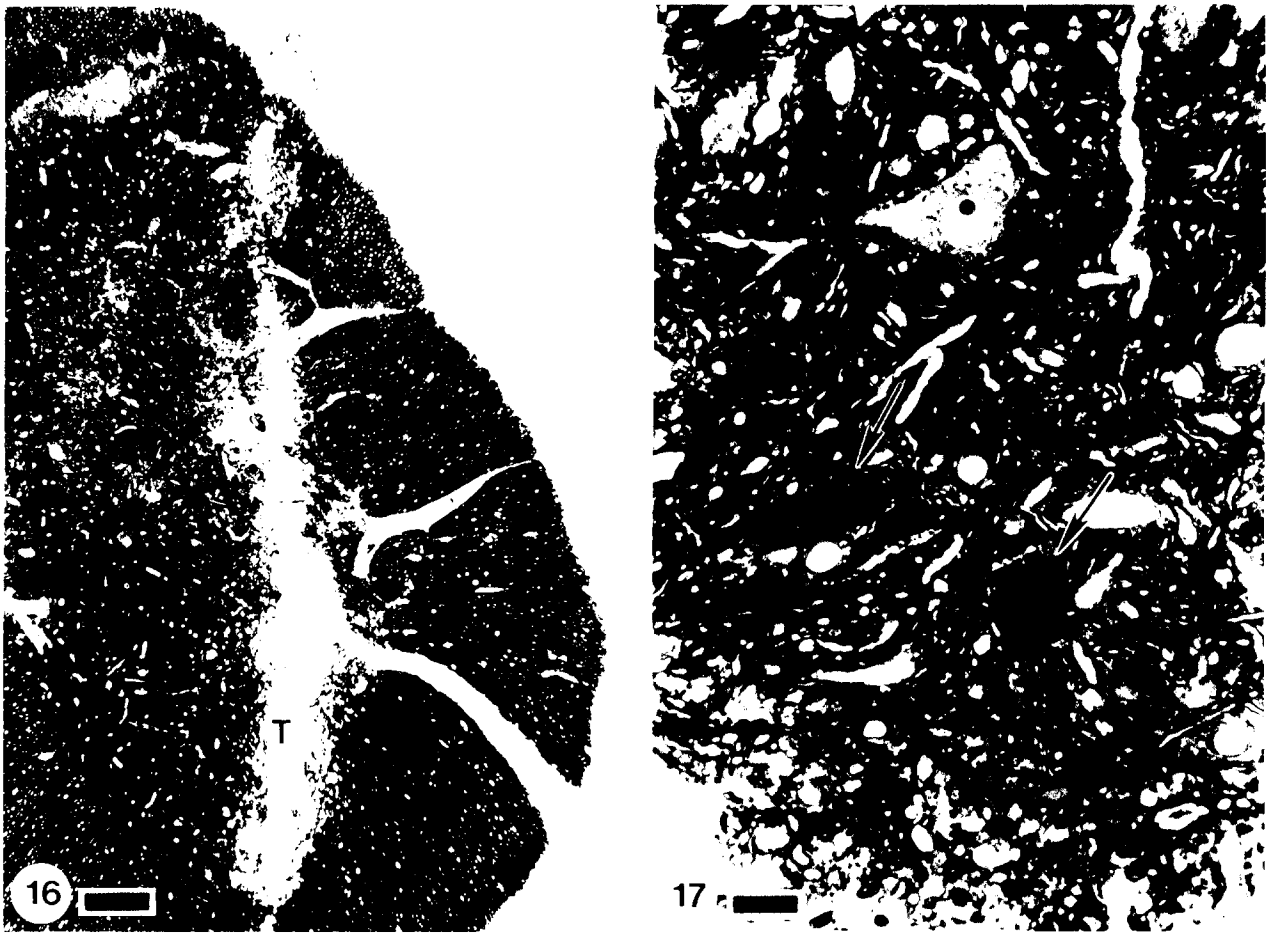


Fig. 16. Animal SP-67. Section through the rostral end of the S2 segment of the spinal cord. The acute electrode track (T) passes through the right dorsal columns and dorsal horn, through the right PPN in the intermediate gray, through the ventral horn and terminates in the ventral columns. Bar = 250 μm.

Fig. 17. Animal SP-67. One μm thick section through the ventral horn at the rostral end of the S2 segment of the spinal cord. Several neurons (arrows) appear dark. Vesiculated cells and axons typical of acute damage are present throughout the micrograph. Bar = 25 μm.